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GB 1375986 A EP 0694308 A1 EP 0474126 A1
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(54) Abstract Title
Stabilisation of Macrolide Compositions

(57) A pharmaceutical composition for oral or parenteral administration comprising a macrolide, preferably a rapamycin or a macrolide of the FK 506 class, is stabilised by the presence of an a mono-, di- or tricarboxylic acid, preferably malonic acid.

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Macrolide compositions

This application is derived from GB 9707483.5.

This invention relates to stabilisation of macrolides.

The term "macrolide" as used herein, refers to a macrocyclic lactone, for example a compound having a 12- membered or larger lactone ring. Of particular interest are the "lactam macrolides", i.e., macrocyclic compounds having a lactam (amide) bond in the macrocycle in addition to a lactone (ester) bond, for example the lactam macrolides produced by microorganisms of the genus Streptomyces such as rapamycin, ascomycin, and FK-506, and their numerous derivatives and analogues. Such lactam macrolides have been shown to have interesting pharmaceutical properties, particularly immunosuppressive and anti-inflammatory properties.

Rapamycin is an immunosuppressive lactam macrolide that is produced by Streptomyces hygroscopicus. The structure of rapamycin is given in Kesseler, H., et al.; 1993; Helv. Chim. Acta; 76: 117. See, e.g., McAlpine, J.B., et al., J. Antibiotics (1991) 44: 688; Schreiber, S.L., et al., J. Am. Chem. Soc. (1991) 113: 7433; US Patent No. 3 929 992. Rapamycin is an extremely potent immunosuppressant and has also been shown to have antitumor and antifungal activity. Its utility as a pharmaceutical, however, is restricted by its very low and variable bioavailability as well as its high toxicity. Numerous derivatives of rapamycin are known. Certain 16-O-substituted rapamycins are disclosed in WO 94/02136, the contents of which are incorporated herein by reference. 40-O-substituted rapamycins are described in, e.g., in US 5 258 389 and WO 94/09010 (O-aryl and O-alkyl rapamycins); WO 92/05179 (carboxylic acid esters), US 5 118 677 (amide esters), US 5 118 678 (carbamates), US 5 100 883 (fluorinated esters), US 5 151 413 (acetals), US 5 120 842 (silyl ethers), WO 93/11130 (methylene rapamycin and derivatives), WO 94/02136 (methoxy derivatives), WO 94/02385 and WO 95/14023 (alkenyl derivatives) all of which

are incorporated herein by reference. 32-O-dihydro or substituted rapamycin are described, e.g., in US 5 256 790, incorporated herein by reference.

Rapamycin and its structurally similar analogues and derivatives are termed collectively as "rapamycins".

Ascomycins, of which FK-506 and ascomycin are the best known, comprise another class of lactam macrolides, many of which have potent immunosuppressive and anti-inflammatory activity. FK506 is a lactam macrolide immunosuppressant that is produced by Streptomyces tsukubaensis No 9993. The structure of FK506 is given in the appendix to the Merck Index, 11th ed. (1989) as item A5. Ascomycin is described, e.g., in US patent 3,244,592. Many derivatives of ascomycin and FK-506 have been synthesized, including halogenated derivatives such as 33-epi-chloro-33-desoxy-ascomycin described in EP 427 680. Ascomycin, FK-506 and their structurally similar analogues and derivatives are termed collectively "ascomycins".

The macrolide may, therefore, be rapamycin or an O-substituted derivative in which the hydroxyl group on the cyclohexyl ring of rapamycin is replaced by $-OR_1$ in which R_1 is hydroxyalkyl, hydroalkoxyalkyl, acylaminoalkyl and aminoalkyl; for example 40-O-(2-hydroxy)ethyl-rapamycin, 40-O-(3-hydroxy)propyl-rapamycin, 40-O-[2-(2-hydroxy)ethoxy]ethyl-rapamycin and 40-O-(2-acetaminoethyl)-rapamycin.

A preferred compound is 40-0-(2-hydroxy)ethyl rapamycin as disclosed in WO 94/09010.

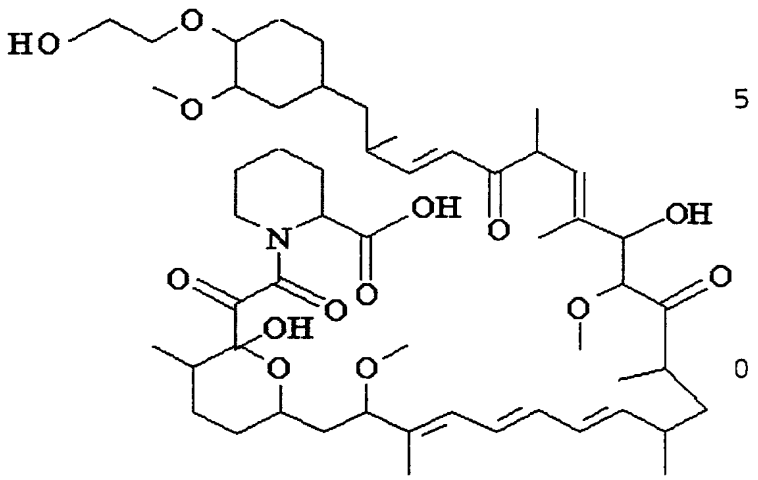
Examples of compounds of the FK 506 class are those mentioned above. They include for example FK 506, ascomycin and other naturally occurring compounds. They include also synthetic analogues.

A preferred compound of the FK 506 class is disclosed in EP 427 680, e.g. Example 66a also known as 33-epi-chloro-33-desoxy-ascomycin. Other preferred compounds are disclosed in EP 465 426, and in EP 569 337, e.g. the compound of Example 71 in EP 569

337.

The present applicants have found that macrolides are unstable upon storage, for example 40-0-(2-hydroxy)ethyl rapamycin, and can undergo a variety of different degradation reactions. Upon storage, for example, of several days, one or more degradation products may be identified, e.g. using HPLC. Although degradation pathways have yet to be identified, the applicants believe that rupture of the macrolide lactone ring may occur.

The present applicants have identified as 40-0-(2-hydroxy)ethyl rapamycin-2,34-secoacid as a main degradation product of 40-0-(2-hydroxy)ethyl rapamycin. 40-0-(2-hydroxy)ethyl rapamycin-2,34-secoacid, referred to hereinafter as secoacid, has the following structure:

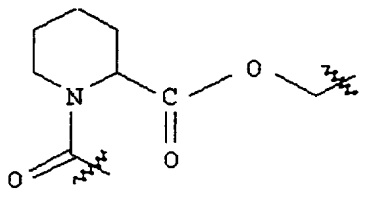


It has now been found that stable compositions containing macrolides may be obtained by formulating the macrolide in an acidic environment. Compositions are understood herein to be stable when the macrolide drug substance remains substantially intact after a period of days or weeks at room temperature (25°C).

In one aspect this invention provides a pharmaceutical composition comprising a macrolide and an acid.

The term macrolide has the meaning as described above.

Preferred macrolides have at least one moiety as follows



Examples are those mentioned above and are preferably rapamycin or 40-0-(2-hydroxy)ethyl rapamycin.

The acid may be lipid soluble and/or ethanol soluble. The acid may be for example a fatty acid, e.g. oleic acid. The acid may be a carboxylic acid, for example a mono-, di- or tri-carboxylic acid, and preferably a mono- or dicarboxylic acid. The acid may comprise one or more hydrophilic groups, e.g. hydroxy groups, and preferably one or two hydrophilic groups. Suitable acids for use in this invention include malonic acid, fumaric acid, maleic acid, D-malic acid, L-malic acid, citric acid, ascorbic acid, succinic acid, oxalic acid, benzoic acid or lactic acid or an acid with a similar pKa, e.g. 2-7. Preferred acids include malonic acid, oxalic acid, citric acid and lactic acid. Malonic acid is more preferred.

The preferred amount of acid may be determined by routine experimentation. The ratio by weight of macrolide to acid in the compositions of this invention may be up to 20:1, for example from 1:5 to 5:1, e.g. 1:1. The acid may be present in an amount of between 0.05% and 5% by weight of the macrolide compositions disclosed in application GB 9707483.5.

The macrolide may be present in an amount of 1 to 15 % by weight of the macrolide compositions disclosed in application GB 9707483.5.

The type of pharmaceutical composition is not critical. It may be solid, but it is preferably liquid. The macrolide may, for example, be formulated into a microemulsion preconcentrate or emulsion preconcentrate as defined in GB 9707483.5, and combined with an amount of acid. The acid-stabilised composition may be administered enterally, e.g orally, e.g. as a capsule or drink solution, or parenterally, e.g. as an infusion concentrate.

In another aspect, this invention provides the use of an acid to stabilise a macrolide in a pharmaceutical composition.

In another aspect, this invention provides a method of stabilising a macrolide in a pharmaceutical composition, which method comprises mixing an acid with the macrolide.

This invention thus allows preparation of stable macrolide compositions. Good drug bioavailability and low variability in inter- and intra-patient dose response may be obtained.

Following is a description by way of example only of macrolide compositions stabilised by an acid.

Example 1

An active agent of the FK 506 class or rapamycin class e.g. 40-0-(2-hydroxy)ethyl rapamycin is made up into a microemulsion preconcentrate having the following composition by weight 2% active compound, 2% malonic acid, lactic acid or famonic acid, 44% Cremophor RH40 26.4% corn-oil mono-, di-, tri-glycerides, 17.6% 1,2 propylene glycol and 10% ethanol.

Stability tests over 3 months showed that a malonic acid composition contained 98% of active agent thereafter and without the malonic acid only 73%.

Examples 2 and 3

Microemulsion preconcentrates are prepared using 40-0-(2-hydroxy)ethyl rapamycin in

Examples 2a and 2b, and rapamycin in Examples 3a and 3b as active agent. In Example 2, the active agent 40-0-(2-hydroxy)ethyl rapamycin is abbreviated to "active agent R".

Intact drug content and main degradation product are determined by HPLC with an analytical error of +/- 2%.

Composition	Example 2a active agent R	Example 2b active agent R malonic acid	Example 3a Rapamycin	Example 3b Rapamycin malonic acid
Cremophor RH 40	44.0 %	43.0 %	41.5 %	40.5 %
Cornoil glyceride	26.3 %	25.7 %	24.8 %	24.2 %
Propylene glycol	17.6 %	17.2 %	16.6 %	16.2 %
Ethanol abs.	10.0 %	10.0 %	15.0 %	15.0 %
DL-α-Tocopherol	0.1 %	0.1 %	0.1 %	0.1 %
active agent R	2.0 %	2.0 %	-	-
Rapamycin	-	-	2.0 %	2.0 %
Malonic acid	-	2.0 %	-	2.0 %
Intact drug content and main degradation product (seco acid) expressed as percentages of amount (HPLC evaluation by external standardization)				
4 weeks at 25°C	86.0% (16.1 %)	99.5 % (0.5 %)	83.5 % (15.4 %)	98.4 % (0.7 %)

Amount of main degradation product is shown in brackets. Main degradation product of rapamycin is referred to as secorapamycin.

The above examples demonstrate that malonic acid exhibits a pronounced stabilizing effect on the degradation of 40-0-(2-hydroxy)ethyl rapamycin and of rapamycin.

Example 4

The composition of Example 2a is mixed with malonic acid at concentrations between 0.05 % and 5% by weight. A highly stabilising effect is observed with malonic acid in the concentration range 0.25 to 0.75% by weight of the composition.

Example 5

A concentrate for infusion is prepared using the following composition:

40-0-(2-hydroxy)ethyl rapamycin	20 mg/ml
Cremophor EL	600mg/ml
citric acid	10mg/ml
ethanol	to 1ml

After 4 weeks storage at 25°C, an active ingredient assay of 99.6% is obtained. This demonstrates that citric acid has a stabilising effect on 40-0-(2-hydroxy)ethyl rapamycin.

In the above Examples 1 to 5 the active agent may replaced by 33-epi-chloro-33-desoxy-ascomycin or by the compound of Example 71 in EP 569 337.

Claims

1. A pharmaceutical composition comprising a macrolide and an acid.

2. A composition as claimed in claim 1 wherein the acid is present in an amount of between 0.05 % and 5% by weight of the composition.

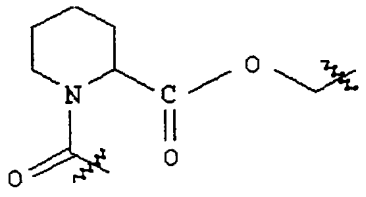
3. A composition as claimed in claim 1 or 2 wherein the ratio by weight of macrolide to acid is up to 20:1, e.g. 1:5 to 5:1.

4. A composition as claimed in any preceding claim wherein the macrolide is an ascomycin or a rapamycin.

5. Use of an acid to stabilise a macrolide against degradation in a pharmaceutical composition.

6. A method of stabilising a macrolide against degradation in a pharmaceutical composition, which method comprises mixing an acid with the macrolide.

7. A method as claimed in claim 6 wherein the macrolide has at least one moiety as follows



8. A method as claimed in claim 6 or 7 wherein the acid is a fatty acid or a mono-, di- or tri-carboxylic acid.

9. A method as claimed in claim 6, 7 or 8 wherein the ratio by weight of macrolide to acid is up to 20:1, e.g. 1:5 to 5:1.

10. A method as claimed in any one of claims 6 to 9 wherein the macrolide is an ascomycin or a rapamycin.

11. A composition as claimed in any one of claims 1 to 4, use or method as claimed in any one of claims 5 to 10 wherein the acid is malonic acid, fumaric acid, maleic acid, D-malic acid, L-malic acid, citric acid, ascorbic acid, succinic acid, oxalic acid, benzoic acid or lactic acid.

12. A composition substantially as herein described with reference to the Examples.

13. Method for stabilising a macrolide against degradation substantially as herein described with reference to the Examples.

The Patent Office

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Application No: GB 9818245.4 **Examiner:** Simon M. Fortt
Claims searched: 1-13 **Date of search:** 20 November 1998

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.P): A5B (BKA)

Int Cl (Ed.6): A61K 31/435, 31/365, 38/13, 47/12.

Other: On-line: CAS-ONLINE

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	GB 1 375 986	(GORDON et al.) whole document particularly p 1, ll 12-17, p 2, ll 54-73, examples	1, 5-6, 8
X, E	EP 0 694 308 A1	(SHISEIDO COMPANY) whole document particularly p 2, ll 49-53, p 3, ll 45-54, examples 4 and 7, experi-ment 1.	1-3, 5-6, 8-9
X	EP 0 474 126 A1	(FUJISAWA PHARMACEUTICAL) p 2, ll 5-6, p 3, l 6 - p 4, l 5, p 6, ll 3-5, example 16.	1-10
X	EP 0 139 891 A2	(B.T.B. INDUSTRIA CHIMICA) whole document particularly p 1, ll 1-18, p 2, ll 9-12, p 14, ll 1-26,	1-3, 5-6, 8-9
X	EP 0 010 437 A2	(ELI LILLY COMPANY) whole document	1-3, 5-6, 8-9, 11
X, P	WO 95/04551 A1	(FUJISAWA PHARMACEUTICAL) see equivalent document US 5, 747, 069 col. 1, ll 44-47, 59-67, col. 3, ll 1-19, 33-54, example 2 (4-6 and 10-12).	1-10

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

The Patent Office

Application No: GB 9818245.4
Claims searched: 1-13

Examiner: Simon M. Fortt
Date of search: 20 November 1998

Category	Identity of document and relevant passage	Relevant to claims
X	WO 90/03793 A1 (MADHOK) whole document	1-3, 5-6, 8-9

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
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